DEVELOPMENT OF EDIBLE MOUTH COOLANTS

by

Richard W. Sroges

IIT Research Institute

Chicago, Illinois

Contract DA-19-129-AMC-96 (N)

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TECHNICAL REPORT
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DEVELOPMENT OF EDIBLE MOUTH COOLANTS

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Richard W. Sroges IIT Research Institute Technology Center Chicago, Illinois

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Food Division
U.S. ARMY NATICK LABORATORIES
Natick, Massachusetts 01760

FOREWORD

This investigation was undertaken to develop edible mouth coolants as complements to food bars high in carbohydrate designed for an austere feeding system to be used by combat troops on special missions.

Such bars are intended to have thirst allaying qualities and to contain ingredients that elicit the oral sensation of coolness. These materials are to be consumed as separate foods or, preferably, are to be incorporated into the food bars.

During the study, several attempts were made to devise a test using rats to screen substances for thirst-alleviating properties; all of these attempts, however, were abandoned.

In conducting the research described in this report, the investigator(s) adhered to the "Principles of Laboratory Animal Care as established by the National Society for Medical Research."

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The Project Officer for the U.S. Army Natick Laboratories was Dr. Karl R. Johnson of the Plant Products Branch, Food Division. Alternate Project Officer was Dr. Maxwell C. Brockmann of the Animal Products Branch, Food Division.

FERDINAND P. MEHRLICH, PhD Director Food Division

APPROVED:

DALE H. SITLING, PhD Scientific Director

W. M. MANTZ
Colonel, OMC
Commanding

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ABSTRACT

This report covers investigations relative to the selection and evaluation of edible substances that could alleviate the oral discomfort associated with thirst. A discussion is also made relative to the rationale of tests that utilize animals to screen substances for thirst-alleviating properties, screening tests that were evaluated, and description of the procedure used in experiments in which human taste panels were utilized to evaluate four substances that appeared to be effective in preliminary tests. Results of the taste panel experiments are also reported.

DEVELOPMENT OF EDIBLE MOUTH COCLANTS

I. INTRODUCTION

The purpose of this study was the selection and evaluation of edible substances that might alleviate the oral discomfort associated with thirst. Previous reports summarized a literature survey, discussed the rationale of tests that utilize animals to screen substances for thirst-alleviating properties, described screening tests that were being evaluated, and described the procedure used in an experiment in which a human taste panel was used to evaluate four substances that appeared to be effective in preliminary tests. This report presents the results of the tastepanel experiment and some additions to the material presented in previous reports.

II. SUBSTANCES TESTED

In the taste-panel experiment, thirst was induced in ten human subjects by having them pedal a stationary bicycle in a hot room. The subjects then rated four test substances, hereafter called substances B, C, D, and E, with regard to their taste qualities and their effects on thirst and the oral sensations accompanying thirst. The subjects also rated the effects of pulverized peppermint Lifesavers, hereafter called substance A. Test substances were to be deemed effective only if they were

significantly more effective than substance A. Substances B, C, D and E were selected on the basis of informal preliminary evaluation by human subjects and were composed of various combinations of monosodium glutamate, sorbitol, mannitol and dextrose hydrate; the exact composition of these substances is shown in Table 1. A small quantity (approximately 0.05 ml per 10 g of dry weight) of oil of peppermint was added to each substance in an attempt to reduce the differences in taste between these substances and substance A.

Table 1
COMPOSITION OF TEST SUBSTANCES

| | Proportion by dry weight* Substance | | | | |
|---|---|---|---|---|--|
| Ingredient | В | C | D | E | |
| Monosodium glutamate Sorbitol Mannitol Dextrose hydrate Citric acid | 0.200 0.500 0.100 0.100 0.100 | 0.222 0.444 0.111 0.111 0.111 | 0.250 0.250 0.125 0.250 0.125 | 0.200 0.200 0.400 0.100 0.100 | |
| | | | | | |

Before addition of oil of peppermint

III. RATINGS OF TEST SUBSTANCES

The subjects rated various effects of the substances on a seven-point scale. The experiment was directed largely to evaluation of the effects of the test substances on thirst, on dryness of the mouth and throat, on the sensation of warmth in the mouth and throat, on irritation of the mouth and throat, and on

coating of the mouth and throat. The means of the subjects' ratings of these effects for each of the substances tested are presented in Table 2.

Table 2

MEAN RATINGS OF EFFECTS OF TEST SUBSTANCES ON THIRST AND ORAL SENSATIONS ASSOCIATED WITH THIRST

| | | Mean Rating Substance | | | | | |
|--|------|-----------------------|------|----------|------|--|--|
| Sensation | A | В | С | <u>n</u> | E | | |
| Thirst | -1.2 | -1.3 | -0.7 | -0.9 | -1:1 | | |
| Dryness of mouth and throat Warmth in mouth and throat Irritation of mouth and throat Coating of mouth and throat and throat | -1.6 | -1.7 | -1.1 | -1.1 | -0.8 | | |
| | -1.1 | 0 | -0.5 | -1.1 | -0.1 | | |
| | -0.4 | -0.2 | 0 | -0.3 | -0.2 | | |
| | -0.2 | 0.8 | 1.1 | 0.3 | 0.7 | | |

In this table a rating of zero indicates absence of any effect, a negative rating indicates a desirable effect (e.g., a reduction in thirst), and a positive rating indicates an undesirable effect (e.g., an increase in thirst), except for the effect on coating of the mouth and throat. Coating of the mouth and throat that was produced by a test substance was considered a desirable effect; hence, in this case, a positive rating is a desirable one. Ratings from -3 to +3 were possible for each of the effects listed. Table 2 shows that, in general, all of the substances produced small effects in the desirable direction. The effects of substance B on thirst and on dryness of the mouth and throat were slightly greater than

those of substance A. All the test substances produced a coating on the mouth and throat, but substance A decreased coating.

In order to test the statistical significance of differences between the mean ratings of given effects, analyses of variance were performed on the ratings of each of the effects listed in Table 2.

Results of the analysis of variance of ratings of the effects of the five test substances on thrist are presented in Table 3.

This analysis revealed no significant differences between substances; however the difference between subjects was significant.

Table 3

ANALYSIS OF VARIANCE OF RATINGS
OF EFFECTS OF TEST SUBSTANCES ON THIRST

| Source of Variation | <u>df</u> | Mean Square | F | P |
|-----------------------------|-----------|----------------|------|------|
| Between subjects | 9 | 3.14 | 2.62 | <.05 |
| Between substances Error | 4 36 | 0.58 1.20 | - | |

Table 4 presents the results of the analysis of variance of ratings of effects of the substances on dryness of the mouth and throat. The results of this analysis are similar to those of the previous one; differences between subjects were significant, but differences between effects of the substances were not.



ANALYSIS OF VARIANCE OF EFFECTS OF TEST SUB-STANCES ON DRYNESS OF MOUTH AND THROAT

| Source of Variation | df | Mean Square | | P |
|---|--------------|----------------------|--------------|------|
| Between subjects Between substances Error | 9 4 36 | 2.67 1.43 0.86 | 3.10 1.66 | <.01 |

Results of the analysis of variance of ratings of effects of the substances on the sensation of warmth in the mouth and throat are presented in Table 5. Again, there were significant differences between subjects but no significant differences in effectiveness of the substances.

Table 5

ANALYSIS OF VARIANCE OF RATINGS OF EFFECTS OF TEST
SUBSTANCES ON SENSATIONS OF WARMTH IN MOUTH AND THROAT

| Source of Variation | df | Mean Square | F | <u>P</u> |
|-------------------------------------|----|----------------|--------------|----------|
| Between subjects Between substances | 9 | 4.39 2.78 | 2.83 1.79 | <.05 |
| Error | 36 | 1.55 | | |

Table 6 contains the results of the analysis of variance of ratings of the effects of the substances on irritation of the mouth and throat. As in the preceding analyses, the differences between subjects were significant but significant differences between substances were not.

Table 6

ANALYSIS OF VARIANCE OF RATINGS OF EFFECTS OF TEST SUBSTANCES ON IRRITATION OF MOUTH THROAT

| Source of Variation | df | Mean Square | <u>_</u> F | р |
|---|--------------|----------------------|------------|------|
| Between subjects Between substances Error | 9 4 36 | 1.84 0.22 0.48 | 3.83 | <.01 |

The results of the analysis of variance of ratings of the effectiveness of the substances in coating the mouth and throat are presented in Table 7. This analysis revealed no significant differences between either subjects or substances.

Table 7

ANALYSIS OF VARIANCE OF RATINGS OF EFFECTS OF TEST SUBSTANCES ON COATING OF MOUTH AND THROAT

| Source of Variation | <u>df</u> | Mean Square | <u>F</u> p |
|---|--------------|----------------------|--------------|
| Between subjects Between substances Error | 9 4 36 | 3.25 2.53 1.86 | 1.75 1.36 |

In summary, initial analyses of the data indicated that test substances B, C, D, and E did not differ significantly from substance A, the peppermint Lifesaver, in their effects on thirst and oral sensations associated with thirst.

In addition to rating the effects of the test substances, the subjects rated the substances for certain taste qualities; the means of these ratings are presented in Table 8. Possible

ratings for the first six qualities listed in the table range from 0 to 6; 0 indicates absence of the quality and 1 to 6 indicate increasing degrees of intensity of the quality. Possible ratings for pleasantness ranged from -3 to +3 with zero indicating neutrality, negative ratings indicating upleasantness, and positive ratings indicating pleasantness.

Table 8

MEAN RATINGS OF TASTE QUALITIES OF TEST SUBSTANCES

| | | Me | ean Ratings | | |
|--------------|-----|----------|-------------|------------|-----|
| | | | Substance | | |
| Quality | A | <u>B</u> | <u>C</u> | <u>D</u> _ | E |
| Coolness | 3.4 | 2.5 | 3.5 | 2.5 | 3.2 |
| Wetness | 3.3 | 3.7 | 3.1 | 3.0 | 3.4 |
| Saltiness | 0.2 | 1.1 | 1.5 | 1.3 | 0.6 |
| Sourness | 0.1 | 1.6 | 1.8 | 1.4 | 0.8 |
| Bitterness | 0.4 | 2.0 | 2.1 | 2.2 | 1.8 |
| Sweetness | 2.6 | 0.8 | 0.6 | 0.9 | 1.3 |
| Pleasantness | 2.2 | -0.8 | -1.0 | -0.4 | 0 |
| | | | | | |

Table 6 shows that none of the substances were rated markedly cool or wet, but substance C was rated slightly cooler than substance A, and substance B was rated slightly wetter than substance A. Substance A was rated sweeter, less salty, less sour, and less bitter than any of the other substances; the fact that substance A was also rated pleasanter than the other substances is probably a reflection of these differences in taste.

As coolness and wetness of a substance may be relevant to its ability to alleviate thirst, the ratings of coolness and wetness

were subjected to analyses of variance in order to determine whether the differences between substances were statistically significant.

The results of the analysis of variance of ratings of coolness of the five substances are presented in Table 9. This
analysis revealed no significant difference between substances;
however, the differences were significant between subjects.

Table 9

ANALYSIS OF VARIANCE OF RATINGS
OF COOLNESS OF TEST SUBSTANCES

| Source of Variation | <u>df</u> | Mean Square | F | р |
|---|--------------|----------------------|------|------|
| Between subjects Between substances Error | 9 4 36 | 6.38 2.37 2.50 | 2.55 | <.05 |

Table 10 contains the results of the analysis of variance of the ratings of wetness of the substances. As in the analysis of ratings of coolness, the differences between the subjects were significant, but the differences between substances were not.





Table 10

ANALYSIS OF VARIANCE OF RATINGS
OF WETNESS OF TEST SUBSTANCES

| Source of Variation | <u>đf</u> | Mean Square | F | <u>p</u> |
|---------------------|-----------|----------------|------|----------|
| Between subjects | 9 | 7.66 | 2.74 | <.05 |
| Between substances | 4 | 0.75 | - | |
| Error | 36 | 2.79 | | |
| | | | | |

An analysis of variance of the ratings of pleasantness of the test substances, the results of which are presented in Table 11, indicated significant differences between subjects. Further analysis of the differences between substances revealed that substance A, the peppermint Lifesaver, had been rated significantly pleasanter than any of the other substances, and that none of the differences between substances B, C, D and E were significant.

Table 11 ANALYSIS OF VARIANCE OF RATINGS OF PLEASANTNESS OF TEST SUBSTANCES

| Source of Variation | <u>df</u> | Mean Square | _ F | <u>p</u> |
|---|--------------|-----------------------|--------------|----------|
| Between subjects Between substances Error | 9 4 36 | 4.04 16.60 1.98 | 2.04 8.38 | <.01 |

With two exceptions, all of the analyses presented thus far revealed significant differences between subjects. The two analyses in which these nearly ubiquitous differences did not appear were the analysis of ratings of coating of the mouth and throat and that of the ratings of pleasantness of the substance.

The two sets of ratings on which the subjects did not differ significantly are also the ones that seem least likely to be directly related to degree of thirst. This suggested the possibility that the observed differences between subjects might have resulted, at least in part, from differences in the degree of thirst induced in different subjects by the experimental procedure. Therefore, in order to determine whether the differences in the degree of thirst induced were significant, the subjects' ratings of this variable were subjected to an analysis of variance. The results, which are presented in Table 12, indicate that the subjects did differ significantly in degree of thirst induced by the experimental procedure.

Table 12

ANALYSIS OF VARIANCE OF RATINGS OF DEGREE OF THIRST INDUCED
BY PEDALLING STATIONARY BICYCLE IN HEATED ROOM

| Source of Variation | df | Mean Square | F | Р |
|---------------------------|---------|----------------|------|------|
| Between subjects Error | 9 36 | 8.48 0.91 | 9.30 | <.01 |

The significant differences found between subjects with regard to the degree of thirst cast some doubt on the validity of the conclusions that follow from some of the previous analyses. This being the case, statistical control of the differences in induced thirst might alter some of the conclusions. Therefore, it was decided that some of the ratings of effectiveness of the test substances should be subjected to analyses of covariance with



the corresponding ratings of induced thirst as covariates. On the hypothesis that the pleasantness of taste of a substance may alter ratings of its effectiveness, the subjects' ratings of pleasantness of the substance were included as a second covariate in the analyses of covariance.

The results of an analysis of covariance with ratings of effects of the test substances on thirst as the criterion variable and the covariates described are presented in Table 13. The differences between the adjusted ratings are not significant. Comparison of the analysis of covariance in Table 13 with the corresponding analysis of variance in Table 3 indicates that the effect of the adjustment resulting from analysis of covariance is to eliminate the significant differences between subjects that were found in the original analysis.

Table 13

ANALYSIS OF COVARIANCE OF RATINGS
OF EFFECTS OF TEST SUBSTANCES ON THIRST

| Sources of Variation | <u>df</u> | Adjusted Mean Square | <u>F</u> | <u>p</u> |
|---|--------------|----------------------|----------|----------|
| Between subjects Between substances Error | 9 4 34 | 2.70 0.66 1.26 | 2.14 | |

Table 14 contains the results of an analysis of covariance similar to the preceding one except that the criterion variable in this case is ratings of the effects of the substances on the sensation of warmth in the mouth and throat. The differences between the adjusted means for subjects and also those between

the adjusted means for substances are significant.

Table 14

ANALYSIS OF COVARIANCE OF RATINGS OF EFFECTS OF TEST SUBSTANCES ON SENSATION OF WARMTH IN MOUTH AND THROAT

| Source of Variation | df | Adjusted Mean Square | <u>F</u> | _p_ |
|---|--------------|----------------------|--------------|--------------|
| Between subjects Between substances Error | 9 4 34 | 4.47 5.73 1.63 | 2.74 3.52 | <.05 <.01 |

Comparison of the results in Table 14 with the results of the corresponding analysis of variance in Table 5 indicates that the effects of the adjustment accomplished by the analysis of covariance are (1) reduction of the level of confidence for the significance of the difference between subjects and (2) revelation of a significant difference between substances that was not revealed by the original analysis. The adjusted mean ratings of the substances can be found in the first row of Table 15.

Substance A, the peppermint Lifesaver, was as effective as, or significantly more effective than, the test substances in cooling the mouth and throat.



Table 15

ADJUSTED MEAN RATINGS OF EFFECTS OF TEST SUBSTANCES
ON SENSATIONS OF DRYNESS AND WARMTH IN MOUTH AND THROAT

| | Adjust | ted Mean Ra | ating | |
|------------|----------|-------------------|---|--|
| | | Substance | | |
| _ <u>A</u> | <u>B</u> | <u> </u> | <u>D</u> | E |
| -1.12 | -0.001 | -0.49 | -1.08 | -0.096 |
| -1.52 | -1.65 | -1.03 | -1.26 | -0.83 |
| | -1.12 | <u>A</u> <u>B</u> | <u>A</u> <u>B</u> <u>C</u> -1.12 -0.001 -0.49 | <u>A</u> <u>B</u> <u>C</u> <u>D</u> -1.12 -0.001 -0.49 -1.08 |

The results of another analysis of covariance similar to the ones described are presented in Table 16. The criterion variable in this analysis was ratings of the effects of the substances on dryness of the mouth and throat. The results of this analysis indicate that the differences between the adjusted means for subjects and also those between the adjusted means for substances are significant.

Table 16

ANALYSIS OF COVARIANCE OF RATINGS OF EFFECTS OF TEST SUBSTANCES
ON DRYNESS OF MOUTH AND THROAT

| Source of Variation | df | Adjusted Mean Square | F | <u>p</u> |
|---|--------------|-----------------------|---------------|--------------|
| Between subjects Between substances Error | 9 4 34 | 3.07 22.78 1.09 | 2.82 20.90 | <.05 <.01 |

comparison of Table 16 with Table 4, which contains the results of the corresponding analysis of variance, indicates that adjustment of the criterion variable by analysis of covariance reduced the level of confidence for the significance of the difference between subjects and revealed a significant difference between substances that was not revealed in the original analysis. The adjusted mean ratings of the effects of the five substances on dryness of the mouth and throat are presented in the second row of Table 15. Only the mean for substance B compares favorably with the mean for substance A; however, the difference between these two means is not statistically significant (t = 0.27).

Thus, even after the ratings have been adjusted to compensate for differences in the degree of thirst induced in different subjects and for differences in the pleasantness of taste of the substances, it is not possible to conclude that any of the four test substances was superior to crushed peppermint Lifesavers in reducing thirst, cooling the mouth and throat, or wetting the mouth and throat.

IV. DURATION OF EFFECTS

An attempt was made to estimate the duration of the effects of the test substances by quizzing the subjects orally after their ingestion of the substances. Most subjects reported no persistent effects. However, one subject reported that substance A produced a coating in the mouth that lasted about 1 hour. Two subjects made similar reports regarding substance C and two regarding substance D.

One subject reported that substance E produced increased salivation, cooling of the mouth, and a coating on the tongue that lasted more than 2 hours. Thus, there is a slight indication that some of the test substances, although no more effective than peppermint Lifesavers, may have more persistent effects, at least for some individuals.

V. RELIABILITY AND VALIDITY OF QUESTIONNAIRE

Five of the ten subjects in the experiment underwent an additional experimental trial during which each received a substance that he had received on an earlier trial. Ratings on relevant items of the questionnaire, i.e., items dealing with the effects of the test substances on thirst and oral sensations and items dealing with taste qualities of the substances, on these two sets of trials were used to estimate the reliability of the questionnaire. The rank order retest reliability coefficient obtained from 12 items for the 5 subjects was .76 (p<.01). Thus, the reliability of the questionnaire appears to be adequate.

In the absence of a good validity criterion, however, the validity of the questionnairs or even of the entire experimental procedure is difficult to assess. It is only possible to state that the procedure and the questionnairs have a reasonable degree of face validity, i.e., that the subjects' ratings on both relevant and irrelevant items are plausible. The significant differences in subjects' ratings of the degree of thirst induced suggest that the procedure could have been improved by the use of some measure

independent of the questionnaire to estimate the degree of thirst induced. If such a measure had been available in this experiment, more nearly equal degrees of thirst probably could have been induced in all subjects.

The fact that there were significant differences between ratings of substance effects by different subjects even after adjustment of the data to correct for differences in induced thirst suggests the existence of differences in the subjects' interpretations of the questionnaire scales. These differences probably could have been reduced by giving the subjects some training in the use of the scales before the experiment. The validity of the questionnaire might also have been increased if a means had been found to more nearly equate the taste qualities of the substances. If these changes are made and, perhaps, if some redundant items are added to the questionnaire so that internal consistency can be evaluated, the method devised for this experiment should prove useful in any future work in this area.

VI. SUPPLEMENTARY COMMENTS ON SCREENING TECHNIQUES USING ANIMALS

During this study, several attempts were made to devise a test using rats to screen substances for thirst-alleviating properties; all of these attempts were abandoned. With the exception of the "two-bottle test," which was abandoned because of failure to replicate the findings of previous investigators, all of these tests were abandoned because it was not possible to train enough animals to make the necessary discriminations





after a reasonable amount of training. The rationale of these attempts, however, still appears to be sound. In fact, selected rats trained in the manner described in previous reports could probably be used for screening purposes. However, the training period would be an extended one, and many of the animals would be useless even after extensive training. Nonetheless, these methods might prove useful in a program involving screening of a very large number of substances; in a small program, it would probably be more economical to use human subjects.

If more economical screening techniques using animals were considered desirable, there would be at least two promising approaches to their development. The methods tried in this study might be adapted to other species; it is quite possible that dogs or monkeys, for example, would learn the necessary discriminations more readily than rats. The alternative is the use of other responses and, probably, other types of apparatus in a test based on a rationale similar to that which has been previously discussed.

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| 13. ABSTRACT | | | |

This report covers investigations relative to the selection and evaluation of edible substances that could alleviate the oral discomfort associated with thirst. A discussion is also made relative to the rationale of tests that utilize animals to screen substances for thirst-alleviating properties, screening tests that were evaluated, and description of the procedure used in experiments in which human taste panels were utilized to evaluate four substances that appeared to be effective in preliminary tests. Results of the taste panel experiments are also reported. /

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